Express Mail Label No.: EK[redacted]16246US
Date of Deposit: July 7, 2000

Attorney Docket No. 21127-501 (formerly 14791-501)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS :	Perrin et al.		
SERIAL NUMBER :	09/314,698	EXAMINER :	Juliet C. Einsmann
FILING DATE :	May 19, 1999	ART UNIT :	1655
FOR :	Micro-Array Based Subtractive Hybridization		

Assistant Commissioner for Patents
Washington, D.C. 20231

Response to Office Action Mailed February 7, 2000

In response to the Office Action ("Office Action") mailed February 7, 2000, please amend the application as follows.

In the Claims

Amend claims 1, 2, 7, 8, 11, 12, 15, 18, 21,and 22 as follows:

1. (Amended) A method for identifying [and isolating] a non-redundant nucleic acid [fragments] in a sample of nucleic acid fragments, the method comprising:

(a) providing a random sample of nucleic acid fragments;

(b) immobilizing the random sample of nucleic acid fragments on a microarray;

(c) hybridizing one or more labeled probes corresponding to previously arrayed or sequenced fragments;

(d) [detecting fragments hybridized to the labeled probes and] identifying at least one immobilized fragment that hybridizes weakly or does not [hybridized or weakly hybridized] hybridize to the labeled probes; and

(e) sequencing a fragment identified in step [(e)] (d) that [was] does not [hybridized] hybridize [or was weakly hybridized] to the labeled probes, thereby identifying a non-redundant nucleic acid fragment in said sample.

2. (Amended) The method of claim ~~1~~, wherein the sample of nucleic acid fragments [comprise] comprises DNA.

7. (Amended) The method of claim 1, wherein the sample of nucleic acid fragments [comprise] comprises RNA.

8. (Amended) The method of claim 1, wherein the nucleic acid fragments in said sample are amplified.

11. (Amended) A method for [enhancing the rate of discovery of expressed mRNA/cDNA sequences and facilitating construction of a "UniGene" set] identifying multiple non-redundant sequences in a random sample of nucleic acid sequences, the method comprising:

(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the random sample of nucleic acids on a solid surface in a microarray format;

(c) hybridizing labeled probes from a DNA source to the immobilized[, microarrayed DNA] nucleic acid fragments;

(d) [detecting DNA fragments hybridized to a labeled probe and] identifying at least one immobilized fragment that hybridizes weakly or does not hybridize [or hybridizes weakly] to the labeled probe;

(e) [determining the [identity of the DNA] sequence of the fragment [by DNA sequencing, hybridization or other analytic approaches] identified in step (d); and

(f) reiterating steps (b) or (c) through (e) [with previously identified sequences in the probe set in order to identify additional sequences and increase the UniGene set], thereby identifying multiple non-redundant sequences in a random ample of nucleic acid sequences.

12. (Amended) A method for [enhancing the rate of discovery of genomic sequences and facilitating isolation of a DNA] identifying multiple nucleic acid fragments corresponding to a whole genome or subregions of interest, the method comprising:

(a) amplifying a random sample of genomic nucleic acid fragments from a whole genome or subregion of interest;

(b) immobilizing the random nucleic acids on a solid surface in a microarray format;

(c) hybridizing labeled probes from a DNA source to the immobilized, microarrayed [DNA] fragments;

(d) detecting DNA fragments which hybridize to [a] the labeled probe;

(e) determining the [identity of the DNA fragment by DNA sequencing [, hybridization or other analytic approaches] sequence of the fragment identified in step (d); and

(f) reiterating steps (b) or (c) through (e) [with previously identified sequences in the probe set in order to identify additional sequences and increase the UniGene set], thereby identifying multiple nucleic acid fragments corresponding to a whole genome or subregions of interest.

15. (Amended) A method for [enrichment and/or isolation of DNA] identifying nucleic acid sequences that are [unique to a population compared to another population] present in different amounts in a first source and a second source, the method comprising:

(a) amplifying and providing a random sample of nucleic acid fragments;

(b) immobilizing the [random] nucleic [acids] acid fragments on a [solid] coated glass surface in a microarray format;

(c) hybridizing labeled probes from a first source and labeled probes from a second source to the immobilized [, microarrayed DNA] nucleic acid fragments;

(d) detecting [DNA] nucleic acid fragments which hybridize to a labeled probe from the first source [or] but which do not hybridize to a labeled probe from the second source; and

(e) determining the [identity] sequence of the DNA fragment [by DNA sequencing , hybridization or other analytic approaches] detected in step (d).

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thereby identifying nucleic acid sequences that are present in different amounts in a first source and a second source.

18. (Amended) A method for [increasing discovery] discovering a [of related DNA sequences] nucleic acid sequence related to a known nucleic acid sequence, the method comprising:

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(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the random nucleic acids on a [solid] coated glass surface in a microarray format;

(c) hybridizing labeled probes to the immobilized [, microarrayed DNA] fragments, [particularly at decreased hybridization stringencies];

(d) detecting [DNA] immobilized fragments which hybridize weakly to a labeled probe;

(e) determining the [identity of the DNA] sequence of the immobilized fragment of step (d) [by DNA sequencing, hybridization or other analytic approaches]; [and]

(f) comparing [DNA] the [sequences] sequence of the immobilized fragment [obtained] to [other available DNA sequences to detect sequences] one or more sequences in a sequence database; and

(g) determining whether said fragment is identical to one or more of said database sequences.

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wherein an immobilized fragment of step (e) which [show] shows homology but [are not] is not identical to sequences in said database is a nucleic acid sequence related to a known nucleic acid sequence.

21. (Amended) A method for [enhancing the rate of removal of] removing undesired sequences from a sample of nucleic acid fragments, the method comprising:

(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the random nucleic acids on a solid surface in a microarray format;

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(c) hybridizing labeled probes [, which are sequences targeted for removal,] to the immobilized [, microarrayed DNA] fragments, wherein the unlabeled probe includes a sequence whose removal is desired from said sample;

(d) [detecting DNA fragments hybridized to a labeled probe and] identifying at least one fragment that hybridizes weakly or does not hybridize [or hybridizes weakly] to the probe ;

(e) determining the [identity of the DNA fragment by DNA sequencing, hybridization or other analytic approaches] sequence of the fragment of step (d); and

(f) reiterating steps (a) or (b) or (c) through (e) [with previously identified sequences in the probe set, as deemed necessary, in order], [to eliminate] thereby eliminating unwanted sequences from the population of fragments.

22. (Amended) A method for identifying changes in copy number of DNA sequences between different sources of nucleic acids, comprising:

(a) amplifying a random sample of nucleic acid fragments [from a given source];

(b) immobilizing the [random nucleic acids] fragments on a [solid] coated glass surface in a microarray format;

(c) hybridizing labeled probes from another source to the immobilized [, microarrayed DNA] fragments;

(d) detecting [DNA] fragments which show absent, significantly lesser, or significantly greater hybridization to a labeled probe relative to fragments from another source; and

(e) determining the identity of the [DNA fragment(s)] fragments identified in (d) [by DNA sequencing, hybridization or other analytic approaches], thereby identifying changes in copy number of DNA sequences between different sources of nucleic acids.

Add the following new claim:

--25. A method for reducing redundancy in a sample of nucleic acid molecules, the method comprising:

(a) providing a heterogeneous sample of nucleic acid molecules;

(b) immobilizing the sample of nucleic acid molecules on a first microarray;

(c) hybridizing to said sample one or more labeled probes corresponding to sequences known to be or suspected of being present in said sample of nucleic acid molecules;

(d) identifying at least one immobilized molecule that hybridizes weakly or does not hybridize to the labeled probes,

(e) providing a probe which specifically recognizes the nucleic acid molecule of (d);

(f) optionally repeating steps (b)-(d) or (b)-(e) using a labeled probe which includes the probe of step (e), thereby reducing redundancy in said sample of nucleic acid molecules.--

REMARKS

Claims 1-25 are pending in the application. Claims 1, 2, 7, 8, 11, 12, 15, 18, 21, and 22 have been amended to more particularly point out and claim the invention, and to correct various informalities. Support for the amendments appears in, *e.g.*, the claims as filed. Additional support for the amendments to claim 11 appears in, at least, page 11, lines 20-21 (discussing non-redundant sequences in a random sample of nucleic acids). The amendment to claims 17, 18, and 22 introducing the "coated glass" language is supported at page 8, lines 23-25. Additional support for the amendments to claims 18 appears at page 6, lines 10-11 (describing sequence databases) and at lines 14-16 (discussing signal-to-noise ratios of less than 0.5). New claim 25 is supported in the specification at, *e.g.*, page 3, line 19 to page 4, line 24. No new matter has been added.

The claims are rejected as indefinite, anticipated, and obvious, on various grounds.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1-24 are rejected as indefinite, on various grounds. The rejection is traversed to the extent it is applied to the claims as amended.

Claims 1-10, 12, and 15-24 are rejected for failing to recite a final process step which meets the preamble to the claim. Claims 1, 12, 15, 17, 21, and 22, from which the remaining claims subject to the rejection depend, have been amended to relate the final recited step to the claim preamble. Accordingly, it is believed this aspect of the rejection can be withdrawn.

Claims 1-10 are rejected because step (e) in claim 1 itself referred to step (e). Claim 1 has been amended so that step (e) refers to step (d).

Claims 1-14 and 18-21 are rejected as indefinite for reciting the phrase "weakly hybridized". This aspect of the rejection is traversed. The Examiner states that the specification does not provide an adequate definition of this phrase, and that it is unclear whether "weakly hybridizes" means some hybridization or no hybridization. However, the specification explains that "weakly" hybridizing refers to a hybridization signal that has a signal-to-noise ratio (S/N) of less than 0.5. While a value of S/N of less than 0.5 may indicate that there is no significant hybridization of a probe molecule to a particular DNA sample, as noted by the specification, one of ordinary skill in the art can readily distinguish a weak hybridization signal from no hybridization signal. For example, weakly hybridizing sequences are frequently used to identify sequences related to, but distinct from, sequences in a target probe. This approach has been used, *e.g.*, to identify new members of gene families. The claim term, therefore, is not indefinite.

Claims 2-9 are rejected as indefinite for reciting "fragment". These claims have been amended to clarify the particular fragment or fragments referred to in the claims.

Claims 11-24 are rejected as indefinite for apparent lack of antecedent basis for the phrase "the DNA fragment" in step (e). The claims have been amended to clarify the antecedent basis of this term.

Claims 18-20 are rejected as indefinite for use of the term "particularly". The claims have been amended to remove this term.

Claims 18-20 are also rejected as indefinite for reciting the phrase "decreased hybridization stringencies". This language has been omitted from the claims.

Claims 18-20 were further rejected because of apparent uncertainty in the relationship between steps (e) and (f) of claim 18. Claim 18 has been amended to clarify that that step (e) requires sequencing the determining the sequence of the immobilized fragment of step (d), and that step (f) requires comparing the sequence of the immobilized fragment to one or more sequences in a sequence database. It is believed these amendments overcome the rejection.

In view of the foregoing amendments and comments, withdrawal of the rejections of rejections for indefiniteness is requested.

Rejections under 35 U.S.C. § 102(b)

Claims 15-17 and 22-24 are rejected as anticipated by Pinkel *et al.*, U.S. Patent No. 5,690,894 ("Pinkel"). The rejection is traversed to the extent it is applied to the claims as amended.

As amended, claim 15, from which depend claims 16 and 17, requires immobilizing nucleic acid fragments on a coated glass surface. Pinkel does not describe immobilizing nucleic acid fragments on a coated glass surface. Instead, it describes biosensor optical fiber arrays that transmit an optical signal from sensor end of the array, on which nucleic acids are immobilized, to a transmission end of the array. (See, *e.g.*, Abstract, FIGS. 1, 4, and col. 7, lines 1-23). Because the surface of the array transmits an optical signal, this reference does not describe a coated glass surface, which is required by the claims. Accordingly, Pinkel does not describe the invention of claim 15, nor of claims 16 and 17.

Claim 22, from which depend claims 23 and 24, is also drawn to a method requiring immobilizing nucleic acid fragments on a coated glass surface. As discussed above, Pinkel fails to describe immobilizing on a glass surface. Accordingly, Pinkel also fails to describe the invention of claim 22, or of claims 23 and 24, for the reasons set forth in the discussion of claim 15, above.

In view of the foregoing comments, withdrawal of the rejections for anticipation is requested.

Rejections under 35 U.S.C. § 103(a)

Claims 1-14 and 21 are rejected as obvious over Kayne et al., (WO98430388) ("Kayne") in view of Gress *et al.*, Mammalian Genome 3:609-612, 1992 ("Gress"). The rejection is traversed.

Claim 1, from which depend claims 2-10, requires that the sequence of a fragment in a population of random, immobilized nucleic acids be determined (see step (e) of claim 1). Neither Kayne nor Gress, alone or in combination, teach or suggest this step. In fact, Kayne

describes a much different method for sequencing a nucleic acid. In Kayne's method, known sequences are attached to a surface and contacted with undefined sequences (see, *e.g.*, Abstract and page 2, lines 10-11). In contrast, the claimed method requires immobilization of random sequences followed by hybridization with a probe whose sequence is known. Kayne also describes a method in which non-hybridized sequences are recovered and sequenced. The claimed invention, however, requires determining the sequence of an immobilized sequence. Thus, for at least these reasons, Kayne teaches a completely different method for identifying a nucleic acid.

The secondary reference, Gress, fails to overcome the deficiencies of Kayne. Gress is cited by the Examiner for teaching a method for hybridization fingerprinting of high-density cDNA library arrays with cDNA pools in which a cDNA library is hybridized to a microarray. Gress does not describe or suggest a method for sequencing an immobilized fragment on an array. Thus the combination of Kayne and Gress fails to make *prima facie* obvious the invention of claim 1. Claims 2-10 depend from claim 1 and are therefore also non-obvious over the cited references.

Independent claims 11 and 12, and 21 all similarly require that the sequence of a fragment in a population of random, immobilized nucleic acids be determined. Thus, for the reasons offered in the comments concerning claim 1, claims 11, 12, and 21 are also non-obvious over the cited references. The remaining claims subject to the rejection, claims 13 and 14, depend from claim 11 and are also non-obvious over the cited references. Accordingly, withdrawal of the rejections for obviousness over the combination of Kayne and Gress is requested.

Claims 18-20 are rejected as obvious over Pinkel in view of Maslyn (U.S. Patent No. 5,953,727). The rejection is traversed.

Claim 18, from which depend claims 19 and 20, requires immobilization on coated glass. As discussed above, Pinkel does not describe immobilization on coated glass. Pinkel also fails to suggest immobilization on coated glass. To the contrary, Pinkel describes immobilizing nucleic acids on a surface that transmits an optical signal through the array to the opposite end of the

array. Thus, to the extent Pinkel describes immobilizing a nucleic acid on a surface through which an optical signal can be transmitted, the reference teaches away from the claimed invention.

The secondary reference, Maslyn, does not overcome the deficiencies of Pinkel. Maslyn is cited for describing a cluster as a group of clones related to each other by sequence homology, and that a cluster can be determined by comparing a sequence against a library or database of sequences. However, Maslyn does not teach or suggest immobilizing a nucleic acid on a coated glass. Accordingly, the combination of Pinkel and Maslyn fail to produce the claimed invention. Therefore, claim 18, as well as claims 19 and 20, is not *prima facie* obvious over the cited references.

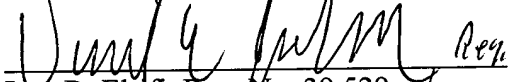
Accordingly, reconsideration and withdrawal of the rejections for obviousness is requested.

CONCLUSION

Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

A petition for an extension of time is enclosed. The Commissioner is hereby authorized to charge any additional fee due with this submission, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 21127-501.

Respectfully submitted,



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Dated: July 7, 2000

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